

1-1-2018

The meiosis-specific Cdc20 family-member Ama1 promotes binding of the Ssp2 activator to the Smk1 MAP kinase.


Gregory Omerza

Chong Wai Tio
Thomas Jefferson University

Timothy Philips
Thomas Jefferson University

Aviva Diamond
Stony Brook University

Aaron M. Neiman
Stony Brook University
Follow this and additional works at: <https://jdc.jefferson.edu/bmpfp>

 Part of the [Medical Cell Biology Commons](#)
See next page for additional authors

[Let us know how access to this document benefits you](#)

Recommended Citation

Omerza, Gregory; Tio, Chong Wai; Philips, Timothy; Diamond, Aviva; Neiman, Aaron M.; and Winter, Edward, "The meiosis-specific Cdc20 family-member Ama1 promotes binding of the Ssp2 activator to the Smk1 MAP kinase." (2018). *Department of Biochemistry and Molecular Biology Faculty Papers*. Paper 131.

<https://jdc.jefferson.edu/bmpfp/131>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Biochemistry and Molecular Biology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Gregory Omerza, Chong Wai Tio, Timothy Philips, Aviva Diamond, Aaron M. Neiman, and Edward Winter

The meiosis-specific Cdc20 family-member Ama1 promotes binding of the Ssp2 activator to the Smk1 MAP kinase

Gregory Omerza^a, Chong Wai Tio^a, Timothy Philips^a, Aviva Diamond^b, Aaron M. Neiman^b, and Edward Winter^{a,*}

^aDepartment of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA 19107;

^bDepartment of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794

ABSTRACT Smk1 is a meiosis-specific MAP kinase (MAPK) in budding yeast that is required for spore formation. It is localized to prospore membranes (PSMs), the structures that engulf haploid cells during meiosis II (MII). Similar to canonically activated MAPKs, Smk1 is controlled by phosphorylation of its activation-loop threonine (T) and tyrosine (Y). However, activation loop phosphorylation occurs via a noncanonical two-step mechanism in which 1) the cyclin-dependent kinase activating kinase Cak1 phosphorylates T207 during MI, and 2) Smk1 autophosphorylates Y209 as MII draws to a close. Autophosphorylation of Y209 and catalytic activity for substrates require Ssp2, a meiosis-specific protein that is translationally repressed until anaphase of MII. Ama1 is a meiosis-specific targeting subunit of the anaphase-promoting complex/cyclosome that regulates multiple steps in meiotic development, including exit from MII. Here, we show that Ama1 activates autophosphorylation of Smk1 on Y209 by promoting formation of the Ssp2/Smk1 complex at PSMs. These findings link meiotic exit to Smk1 activation and spore wall assembly.

Monitoring Editor

Mark J. Solomon
Yale University

Received: Jul 26, 2017

Revised: Oct 27, 2017

Accepted: Oct 31, 2017

INTRODUCTION

Meiosis is a specialized form of cell division in which a diploid cell undergoes one round of DNA replication followed by two rounds of chromosome segregation. While meiosis leads to the formation of egg and sperm cells in vertebrates, it leads to the formation of spores in yeast. In many ascomycetes, including *Saccharomyces cerevisiae*, meiosis is connected to spore formation by prospore membranes (PSMs) (Neiman, 1998). These double-membraned structures engulf the products of meiosis II (MII) as they are being formed and pinch off to generate four haploid cells within the mother cell. Spore wall assembly takes place within and around

PSMs, giving rise to multilayered spore walls that protect the haploids from environmental insults (Neiman, 2005, 2011).

Smk1 is a meiosis-specific MAP kinase (MAPK) that is specifically required for spore morphogenesis (Krisak *et al.*, 1994). The *SMK1* promoter is induced by the middle-meiotic transcription factor, Ndt80, as cells exit meiotic prophase (G2) and enter meiosis I (MI) (Pierce *et al.*, 1998; Ahmed *et al.*, 2009; Shin *et al.*, 2010). The Smk1 protein is activated in a noncanonical pathway that is coordinated with the subsequent stages in meiosis. First, the cyclin-dependent kinase (CDK)-activating kinase Cak1 phosphorylates residue T207 in Smk1's activation loop (Wagner *et al.*, 1997; Whinston *et al.*, 2013). Phosphorylation of T207 is insufficient to activate the kinase, and Smk1 remains catalytically inactive until bound by the Ssp2 activator protein later in the pathway (Tio *et al.*, 2017). Similar to *SMK1*, *SSP2* is controlled by an *NDT80*-inducible promoter (Chu *et al.*, 1998). However, in contrast to *SMK1*, *SSP2* mRNA is translationally repressed until MII, when the meiosis-specific CDK-like kinase Ime2 triggers its translation (Brar *et al.*, 2012; Berchowitz *et al.*, 2013; Tio *et al.*, 2015). Ssp2 localizes to PSMs, where it binds Smk1 and activates the kinase (Tio *et al.*, 2015, 2017). Smk1 then autophosphorylates Y209, further increasing catalytic activity. Thus, this pathway delivers an activated MAPK to the subcellular location where it is needed (the PSM) at a defined stage in meiosis (exit from MII).

This article was published online ahead of print in MBoc in Press (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E17-07-0473>) on November 8, 2017.

*Address correspondence to: Edward Winter (edward.winter@jefferson.edu).

Abbreviations used: APC/C, anaphase-promoting complex/cyclosome; CDK, cyclin-dependent kinase; GFP, green fluorescent protein; GST, glutathione S-transferase; HA, hemagglutinin; KAD, kinase-activating domain; MAPK, MAP kinase; MI, meiosis I; MII, meiosis II; PSM, prospore membrane; RRM, RNA-recognition motif; TCA, trichloroacetic acid; TD, targeting domain.

© 2018 Omerza *et al.* This article is distributed by The American Society for Cell Biology under license from the author(s). Two months after publication it is available to the public under an Attribution–Noncommercial–Share Alike 3.0 Unported Creative Commons License (<http://creativecommons.org/licenses/by-nc-sa/3.0>).

“ASCB®,” “The American Society for Cell Biology®,” and “Molecular Biology of the Cell®” are registered trademarks of The American Society for Cell Biology.

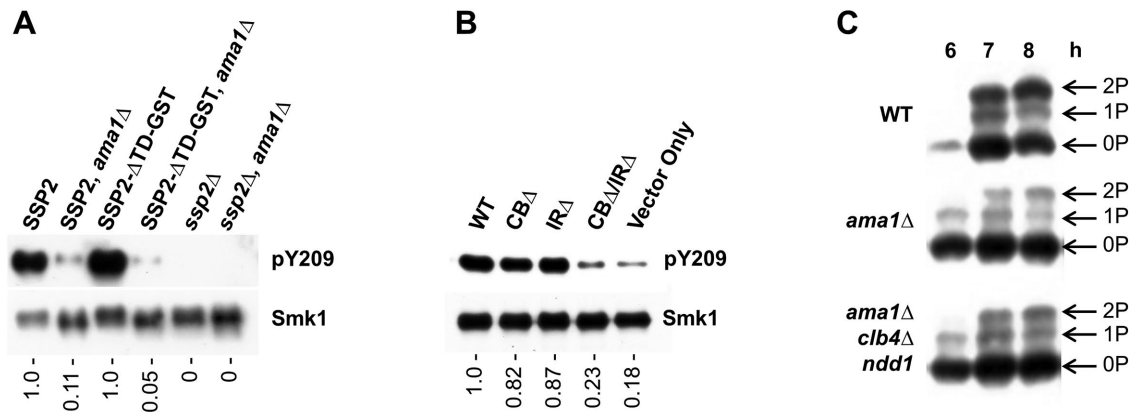


FIGURE 1: APC/C^{Ama1} promotes Smk1 autophosphorylation on Y209. (A) *SMK1-HH* strains containing the indicated mutations were harvested after most cells had completed MII (9 h postinduction; greater than 75% of cells had completed MII in all cases). Smk1-HH was purified under denaturing conditions and quantitated. Comparable amounts of Smk1-HH were then analyzed using a phospho-specific antisera (pY209) and an HA antibody (Smk1). Quantitation of the pY209:Smk1 band ratios are shown below each lane with the values of the *AMA1* strains (first and third lanes from the left) set at 1.0 to facilitate comparison with the *ama1*Δ strains (second and fourth lanes from left). (B) *ama1*Δ *SMK1-HH* cells containing the indicated *AMA1* plasmids were collected at 9 h postinduction, Smk1-HH was purified, and pY209 levels were assayed as in A. (C) *SMK1-HA* cells that are otherwise wild type (WT), *ama1*Δ, or *ama1*Δ *clb4*Δ *ndd1* were transferred to sporulation medium and collected at the indicated time points (h). Total protein was analyzed by phostag-acrylamide gel electrophoresis and immunoblot analyses using an HA antiserum. Unphosphorylated (0P), monophosphorylated (1P), or doubly phosphorylated (2P) forms of Smk1 are indicated. The fraction of Smk1 that is doubly phosphorylated at the 8-h time point in wild-type, *ama1*Δ, and *ama1*Δ *clb4*Δ *ndd1* cells in this experiment is 47, 3, and 8%, respectively.

Ssp2 consists of an N-terminal targeting domain (TD) that localizes Ssp2 to the PSM and a kinase-activating domain (KAD). The KAD contains two motifs that have been annotated as RNA-recognition motifs (RRMs) based on sequence similarity. However, an interaction between Ssp2 and RNA has not been reported. Although Ssp2 lacking the TD (Ssp2-ΔTD) is delocalized, it can activate Smk1 autophosphorylation and promote spore wall assembly (Tio *et al.*, 2015). While the translational induction of Ssp2 couples Smk1 activation to anaphase II, other mechanisms may also temporally connect Smk1 activation to completion of the meiotic program.

Previous studies have shown that Ama1, a meiosis-specific targeting subunit of the anaphase-promoting complex/cyclosome (APC/C) E3 ubiquitin ligase, positively regulates Smk1 (McDonald *et al.*, 2005). APC/C^{Ama1} is required for multiple steps in meiotic development, and the *ama1*Δ meiotic phenotype is complex (Cooper *et al.*, 2000; Oelschlaegel *et al.*, 2005; Penkner *et al.*, 2005; Torres and Borchers, 2007; Diamond *et al.*, 2009; Tan *et al.*, 2011, 2013; Okaz *et al.*, 2012). For example, Ama1 targets both Ndd1, a transcriptional regulator that controls progression of the mitotic cell cycle, and Clb4, a mitosis-specific M-phase cyclin, for ubiquitin-dependent degradation during meiotic prophase. Destruction of these mitotic factors is essential for the recombination checkpoint to block meiosis in the presence of persistent recombination intermediates and thus for high-fidelity chromosome segregation (Okaz *et al.*, 2012). Further, Ama1 targets the APC/C targeting subunit Cdc20 for destruction (Tan *et al.*, 2011). As cells complete the meiotic divisions, APC/C^{Ama1} targets another component of the PSM named Ssp1 for destruction in what has been proposed to be a regulatory step in PSM closure (cellularization of the haploids within the mother cell) (Maier *et al.*, 2007; Diamond *et al.*, 2009; Paulissen *et al.*, 2016). In addition, *ama1*Δ cells disassemble meiotic spindles inefficiently, suggesting that Ama1 promotes exit from MII (Okaz *et al.*, 2012). Although strain-specific differences in the terminal phenotype have

been reported, in the SK1 (hypersporulating) strain background, *ama1*Δ cells block the meiotic program with four nuclear masses surrounded by PSMs. However, these PSMs fail to pinch off and spore wall assembly is undetectable (Diamond *et al.*, 2009).

Although Ama1 has been implicated as an activator of Smk1, the mechanism of activation has not been determined (McDonald *et al.*, 2005). Here, we show that Smk1 and Ssp2 are produced in a timely manner and localize to the PSM in *ama1*Δ cells. However, Smk1 and Ssp2 do not form a complex in *ama1*Δ cells, and the Y209 phosphorylated fraction of Smk1 is reduced. In the *ama1*Δ background, Smk1 and Ssp2 are hyperstabilized, and they accumulate and persist at the PSM. Taken together, these data demonstrate that Smk1 and Ssp2 are poised at the PSM, but their interaction and Smk1 activation require Ama1.

RESULTS

APC/C^{Ama1} is required for Smk1 autophosphorylation

For determination of whether Ama1 influences the autophosphorylation of Smk1 on Y209, Smk1 tagged on its C-terminus with polyhistidine and a hemagglutinin (HA) epitope (Smk1-HH) was purified under denaturing conditions from postmeiotic *ama1*Δ and wild-type cells. The relative amounts of phosphorylated Y209 in these samples were analyzed using a phosphospecific antiserum. The fraction of Smk1 phosphorylated on Y209 was substantially decreased in the *ama1*Δ strain (by 89 and 91% in two separate experiments) (Figure 1A). Ama1 contains conserved APC/C interaction segments termed CB and IR motifs. While *ama1-CB*Δ and *ama1-IR*Δ are partially able to support spore wall formation, the double *ama1-CB*Δ,*IR*Δ mutant is phenotypically indistinguishable from the *ama1*Δ mutant (Tan *et al.*, 2011). For testing whether Ama1 regulates Smk1 autophosphorylation in an APC/C-dependent manner, Smk1-HH purified from *ama1*Δ cells containing *ama1-CB*Δ, *ama1-IR*Δ, or *ama1-CB*Δ,*IR*Δ plasmids was analyzed using the pY209 antiserum.

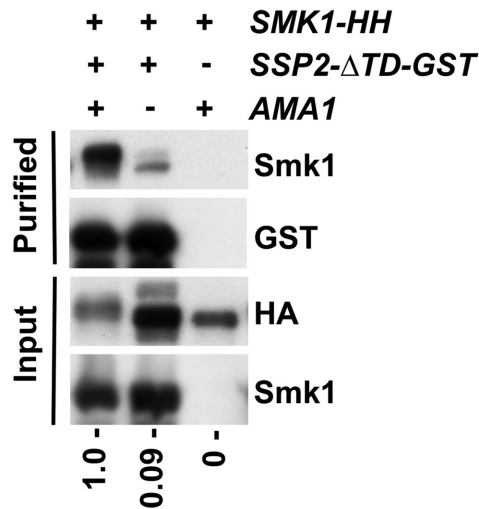


FIGURE 2: The interaction of Ssp2 and Smk1 requires *AMA1*. *SMK1-HH* *SSP2-ΔTD-GST* cells with or without *AMA1* as indicated were collected at 9 h postinduction, when more than 80% of cells had completed MII. Ssp2-ΔTD-GST was purified using glutathione resin, and the input and purified samples were analyzed using an HA antibody (Smk1) or a GST antibody as indicated. The strain lacking *SSP2-ΔTD-GST* serves as a negative control. The values below each lane show the ratios of the HA:GST signals in the purified fractions (above) divided by the ratios of the HA:GST signals in the input samples (below), with the value in the *AMA1* *SSP2-ΔTD-GST* (leftmost lanes) set at 1.0 for comparison.

While plasmid-encoded *AMA1* is not as effective as *AMA1* expressed from its natural chromosomal location in promoting Smk1 autophosphorylation, *AMA1* plasmids did increase the fraction of autophosphorylated Smk1 in *ama1Δ* cells. Both the *ama1-CBΔ* and the *ama1-IRΔ* plasmids also increased the fraction of Smk1 that was autophosphorylated on Y209, while the *ama1-CBΔ/IRΔ* mutant plasmid had little or no effect (Figure 1B). These findings indicate that the role of Ama1 in activating Smk1 autophosphorylation is dependent on binding the APC/C.

We previously showed that phosphorylation of Smk1's activation loop can be analyzed using electrophoresis through gels containing phostag-acrylamide (Whinston *et al.*, 2013). Based on this assay, the fraction of Smk1 that is doubly phosphorylated (slowest migrating band) is substantially reduced in *ama1Δ* cells, consistent with the pY209 phosphospecific immunoassay (Figure 1C). It has previously been shown that Ama1 is required for the degradation of the Clb4 cyclin and the Ndd1 transcription factor in meiotic G2 and that increased levels of these mitotic factors in *ama1Δ* cells cause meiosis to proceed in a dysregulated manner (Okaz *et al.*, 2012). We used phostag-acrylamide electrophoresis to monitor Smk1 phosphorylation in *ama1Δ clb4Δ* cells, in which the sole source of Ndd1 (required for vegetative growth), was provided by the *SSC1* mitosis-specific promoter (*SSC1pr-NDD1*). The fraction of doubly phosphorylated Smk1 in the *ama1Δ clb4Δ SSC1pr-NDD1* mutant was comparable with that observed in the *ama1Δ* single mutant (Figure 1C). These findings suggest that increased levels of Clb4 and Ndd1 in the *ama1Δ* background do not account for the reduced level of autophosphorylated Smk1.

Ssp2 forms a complex containing autophosphorylated Smk1 that is localized to the PSM. While Ssp2 lacking its N-terminal TD (Ssp2-ΔTD) is delocalized, this truncated form of Ssp2 is functional, and

ssp2-ΔTD cells form spores that are indistinguishable from wild-type spores (Tio *et al.*, 2015). We found that the end-stage sporulation phenotypes of *ama1Δ* and *ama1Δ ssp2-ΔTD* cells are indistinguishable (both strains form meiosis-positive asci that are sporulation defective). *ama1Δ* reduced Smk1 autophosphorylation in the *ssp2-ΔTD* background (to 95 and 92% in two separate experiments) (Figure 1A, third and fourth lanes from the left). These findings demonstrate that Ama1 controls the activation of Smk1 even when the Ssp2 activator is delocalized.

AMA1 promotes formation of the Smk1-Ssp2 complex at the PSM

To investigate whether *AMA1* is required for formation of the Ssp2/Smk1 complex, we used glutathione *S*-transferase (GST)-tagged forms of Ssp2. Ssp2-ΔTD-GST is more soluble than full-length Ssp2-GST, and this facilitates analyses of the Ssp2/Smk1 interaction. Ssp2-ΔTD-GST was purified from *ama1Δ* and wild-type cells expressing *SMK1-HH*. The amount of Smk1-HH that copurified with Ssp2-ΔTD-GST in postmeiotic *ama1Δ* cells was $7 \pm 3\%$ of that seen in wild type ($n = 3$) (Figure 2). These data indicate that Ama1 controls the Smk1 pathway by promoting the interaction of Ssp2 and Smk1.

Smk1 localizes to PSMs during anaphase II (Tio *et al.*, 2015). Although Ssp2 is found almost exclusively at PSMs and may play a role in retaining Smk1 at PSMs, Smk1 localizes to PSMs in the absence of *SSP2*. We transformed wild-type and *ama1Δ* strains containing the HTB2-mCherry histone marker with multicopy plasmids containing *SSP2*-green fluorescent protein (*SSP2-GFP*) or *SMK1*-GFP and monitored cells that were completing meiosis by fluorescence microscopy (Figure 3). Although Smk1-GFP and Ssp2-GFP fluorescence was observed in a fraction of these cells that varied from experiment to experiment (a consequence of plasmid loss), the fractions of wild-type and *ama1Δ* cells with detectable Smk1-GFP and Ssp2-GFP within a given experiment were identical, and intensities of the fluorescence signals in the two strains as MII was being completed were indistinguishable. Importantly, as MII was being completed, Smk1-GFP and Ssp2-GFP fluorescence was tightly localized to PSMs in more than 95% of the fluorescent wild-type and *ama1Δ* cells. Taken together, these data indicate that Ama1 exerts its influence on the Smk1 signaling pathway after Ssp2 and Smk1 are localized at the PSM.

Ama1 and Ssp2 regulate Smk1 stability

Previous work suggested that Ssp2 not only activates Smk1 but that it also has a stabilizing influence on Smk1 as cells complete meiosis (Whinston *et al.*, 2013). To investigate the interplay between Ssp2 and Ama1 in controlling Smk1 stability, we monitored Smk1-HA in *ama1Δ* and *ssp2Δ* cells at various times after transfer to sporulation medium (Figure 4).

Smk1 accumulation in wild-type cells is biphasic. The first phase starts around 5 h postinduction, when many cells are in pachytene, the late stage of meiotic G2 when Ndt80 is induced (Whinston *et al.*, 2013). Smk1 accumulation continues during the 5- to 6.5-h interval as most cells are carrying out MI. This first phase of Smk1 accumulation will be referred to as "meiotic." During the 6.5- to 8-h interval, as cells complete MII and Ssp2 is translated, the amount of Smk1-HA further increases. This second phase of Smk1 accumulation will be referred to as "postmeiotic." Previous studies have shown that Ssp2 translation and Smk1 autophosphorylation occur during the postmeiotic phase of Smk1 accumulation (Whinston *et al.*, 2013; Tio *et al.*, 2015), which is undetectable in *ssp2Δ* cells. The postmeiotic Smk1 accumulation was also muted in the *ama1Δ* mutant. One

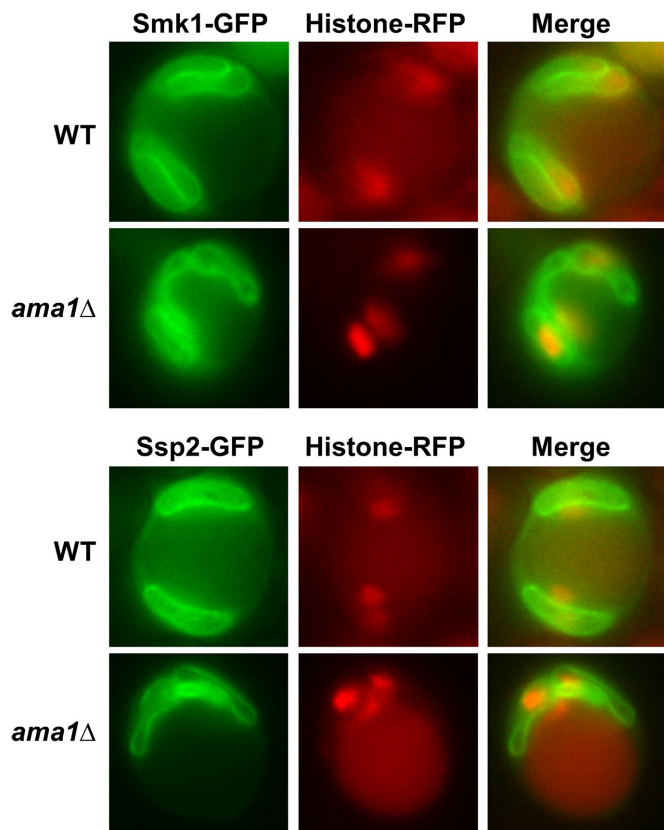


FIGURE 3: Smk1 and Ssp2 localize to the PSM in *ama1Δ* cells. Wild-type and *ama1Δ* cells containing the nuclear HTB2-mCherry marker (strains LH902 and TPY10 respectively) transformed with multicopy plasmids containing either *SMK1-GFP* (top) or *SSP2-GFP* (bottom) (see Table 2) were transferred to sporulation media and viewed by fluorescence microscopy as they completed meiosis.

explanation for the absence and reduction of postmeiotic accumulation of Smk1 in the *ssp2Δ* and *ama1Δ* backgrounds, respectively, is that Ssp2 binding protects Smk1 that is translated in postmeiotic cells from degradation.

Smk1 exists transiently during sporulation, and by 24 h postinduction, when the program has been completed, Smk1 was barely detectable in wild-type and *ssp2Δ* cells. In contrast, high levels of Smk1 were present at the 24-h time point in cells lacking *AMA1* (Figure 4, A and B). Similar to Smk1, the level of Ssp2 declines in the wild type as spore wall assembly is taking place. Also similar to Smk1, Ssp2 is stabilized in the *ama1Δ* background, with high levels of Ssp2 present at the 24-h time point (Figure 4, A and C). These findings suggest that the developmentally regulated degradation of Smk1 and Ssp2 are linked. We also note that, while the decline in Smk1 by 24 h in wild-type cells is almost complete, the decline in amount of Ssp2 is partial, and that Ssp2 that is present at 24 h postinduction migrates through electrophoretic gels more slowly than the Ssp2 that is present earlier in the program. These observations raise the possibility that Ssp2 has a function late in the program that is independent of Smk1.

Consistent with the immunoblot data, Smk1-GFP fluorescence at the 24-h time point was detected at a much higher level in the *ama1Δ* background than in the wild type (Figure 4D). The fluorescence in the 24-h *ama1Δ* cells was located throughout the ascoplasm in aggregated and misshapen structures that are likely PSM

remnants. Taken together, these findings suggest that, while *Ama1* stabilizes Smk1 by promoting association with Ssp2 as MII is being completed, it is also required for the developmentally regulated elimination of Smk1 after key steps in spore wall formation have been completed. Smk1 could be persistent in the *ama1Δ* mutant at late time points (24 h postinduction), because *ama1Δ* blocks the program before the point when Smk1 degradation is induced and/or because *Ama1* plays a direct role in eliminating Smk1 once key steps in the program have been completed.

Ssp2 is persistently phosphorylated when Smk1 is inactive

It has previously been shown that the Ssp2 that is translated during anaphase II is rapidly phosphorylated and that Smk1 autophosphorylation and Ssp2 dephosphorylation occur around the same time (Whinston *et al.*, 2013). These observations led to the suggestion that the dephosphorylation of Ssp2 plays a role in triggering Smk1 activation. We analyzed the phosphorylation of Ssp2-MYC by phostag-acrylamide electrophoresis in wild-type and *ama1Δ* cells at various times postinduction (Figure 5). When Ssp2-Myc is first produced (6.5 h), two major forms are present in both wild-type and *ama1Δ* cells. The electrophoretic resolution of the major slower-migrating form (labeled Ssp2-P in Figure 5) from the faster-migrating form of Ssp2 requires the inclusion of phostag-acrylamide in the electrophoretic gel, indicating that Ssp2-P is phosphorylated. While the relative amount of Ssp2-P decreases substantially as wild-type cells progress through meiosis, the decrease in the Ssp2-P:Ssp2 ratio is not observed in *ama1Δ* cells. These findings indicate that Ssp2 is inefficiently dephosphorylated in *ama1Δ* cells.

Cdc20 family members are known to promote the dephosphorylation of cell cycle regulatory proteins that have been phosphorylated by CDKs (Pesin and Orr-Weaver, 2008). The persistent phosphorylation of Ssp2 in *ama1Δ* cells might prevent Ssp2 and Smk1 from forming a stable complex or it could be a consequence of a failed Ssp2/Smk1 interaction. Previous studies have demonstrated that a catalytically inactive form of Smk1 (Smk1-K69R) binds to Ssp2-GST indistinguishably from wild-type Smk1 (Tio *et al.*, 2017). We next tested Ssp2-MYC phosphorylation in *smk1Δ* and *smk1-K69R* backgrounds containing and lacking *AMA1*. Ssp2 is persistently hyperphosphorylated in all of the mutants tested (Figure 5B). These observations suggest that activated Smk1 is required to promote the dephosphorylation of Ssp2 and that the dephosphorylation of Ssp2 is a consequence of Smk1 activation and not a regulatory step that controls formation of the Ssp2/Smk1 complex. We also assayed Ssp2-ΔTD-Myc in wild-type and *ama1Δ* cells using phostag-acrylamide electrophoresis and found no evidence that this truncated protein was differentially phosphorylated at different stages of meiosis (Figure 5C). These observations are consistent with *Ama1* controlling Ssp2/Smk1 complex formation through a mechanism that is independent of Ssp2 dephosphorylation.

An Ssp2 mutant that fails to bind Smk1 is persistently phosphorylated

It has previously been shown that deletions that eliminate residues at Ssp2's carboxy terminus eliminate the ability of Ssp2 to form a complex with Smk1 (Tio *et al.*, 2015). During the course of our studies, we identified several missense mutations in the C-terminal coding region of *SSP2* that cause a sporulation-defective phenotype (unpublished data). One of these defective alleles contains F to A changes at residues 307 and 327 of the protein (referred to later as *ssp2-F/A*). These substitutions change aromatic amino

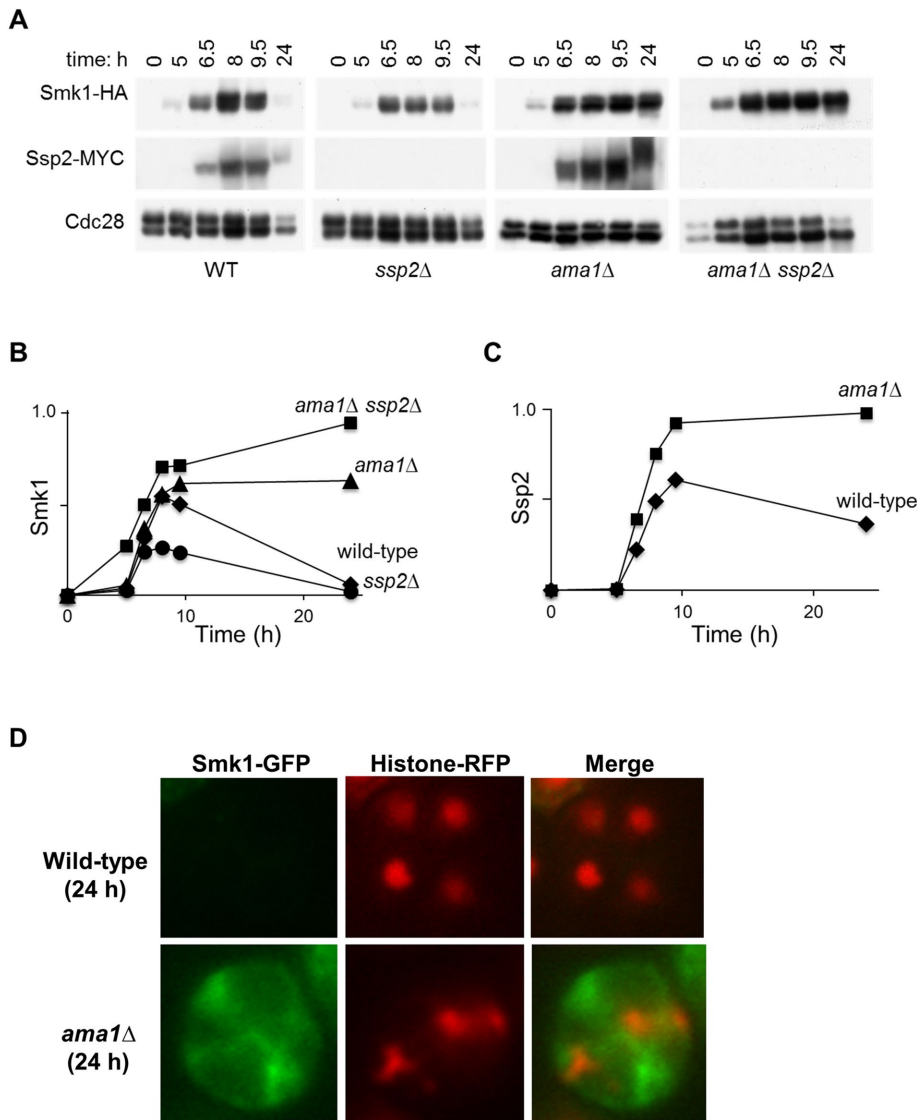


FIGURE 4: *AMA1* and *SSP2* influence Smk1 accumulation/stability. (A) *SMK1-HA* strains deleted for *AMA1* and/or *SSP2* were collected at the indicated times, and Smk1-HA, Ssp2-Myc, or Cdc28 (a control for protein loading) were monitored by immunoblot analyses. Meiosis was scored for each sample, and although MI was advanced slightly in cells lacking *AMA1*, MII had been completed in more than 70% of cells by the 8-h time point in all cases. (B) Quantitation of the Smk1-levels normalized for Cdc28 (lower member of the doublet) from A. (C) Quantitation of the Ssp2 levels normalized for Cdc28 from A. (D) Wild-type and *ama1Δ* cells containing the nuclear marker HTB2-mCherry and a *SMK1-GFP* plasmid were imaged by fluorescence microscopy at 24 h postinduction when most of the Smk1 had been degraded in wild-type cells and Smk1-GFP is barely detectable (equivalent exposures shown for comparison).

acids in the C-terminal RRM-like motif of Ssp2 that align with residues in well-studied RRMs that interact with cognate RNAs (Daubner et al., 2013). Phos-tag acrylamide electrophoretic analyses revealed that the F/A substitutions prevent the postmeiotic accumulation of autophosphorylated Smk1, similar to *ssp2Δ*, and that Ssp2-F/A is persistently phosphorylated and comigrates with Ssp2-P (Figure 6A). While wild-type Ssp2-ΔTD-GST formed a stable complex with Smk1, Ssp2-ΔTD-F/A-GST did not (Figure 6B). Nevertheless, the F/A form of Ssp2 fused to GFP localized to PSMs (Figure 6C). Thus, the behavior of Ssp2-F/A in otherwise wild-type cells resembles that of wild-type Ssp2 in the *ama1Δ* mutant. Collectively, these data suggest that, although hyperphosphorylated

Ssp2 is correlated with uncomplexed Ssp2 in meiotic cells, dephosphorylation of Ssp2 is a downstream consequence of Smk1 activation and not a mechanism used by Ama1 to regulate formation of the Ssp2/Smk1 complex.

DISCUSSION

Metaphase II in *S. cerevisiae* is followed in rapid succession by anaphase II, exit from MII, PSM encapsulation of the haploid, and spore wall assembly. The APC/C E3 ubiquitin ligase functions as a major driver of these events through the use of the Cdc20, Cdh1, and Ama1 targeting subunits (Cooper and Strich, 2011). The meiosis-specific targeting subunit Ama1 plays a particularly prominent role in controlling exit from MII, PSM closure, and spore wall assembly. In this study we have shown that Ama1 triggers activation of the Smk1 MAPK by regulating the interaction of Ssp2 and Smk1 at the PSM.

SMK1 and *SSP2* are both transcriptionally induced by Ndt80 as cells exit meiotic G2 and enter MI (Ahmed et al., 2009; Shin et al., 2010). While *SMK1* mRNA is translated shortly after transcription, *SSP2* is a member of a subset of genes whose mRNAs are repressed until the meiosis-specific Ime2 CDK-like kinase triggers their translation at MII by phosphorylating the Rim4 translational repressor (Brar et al., 2012; Berchowitz et al., 2013, 2015; Tio et al., 2015). This mechanism provides the first-level temporal control that prevents Smk1 activation until the MII spindle is present and PSM outgrowth is occurring. It has previously been shown that Smk1 and Ssp2 localize to the growing PSM independently (Tio et al., 2015). In the present study, we show that *ama1Δ* decreases the activation state of Smk1. Although both Smk1 and Ssp2 localize to the PSM, the interaction between Smk1 and Ssp2 is substantially reduced in the *ama1Δ* background. These findings suggest the Smk1 is poised for activation at the PSM as MII is taking place and that an Ama1-dependent step promotes formation of the Ssp2/Smk1 complex, thereby triggering activation of the kinase. Ama1 also promotes exit from MII (disassembly of the anaphase II spindle), and it is required for PSM closure (Diamond et al., 2009; Okaz et al., 2012). These observations suggest that Ama1 couples these events to Smk1 activation, thereby delaying spore wall assembly until meiosis has been completed and cellularization of the four haploid cells has taken place within the mother cell.

Ssp2 is hyperphosphorylated shortly after it is translated (during MII), and it accumulates in a dephosphorylated form around the time that Smk1 autophosphorylation takes place. On the basis of these findings, we previously hypothesized that the dephosphorylation of Ssp2 is required for Smk1 activation (Whinston et al., 2013).

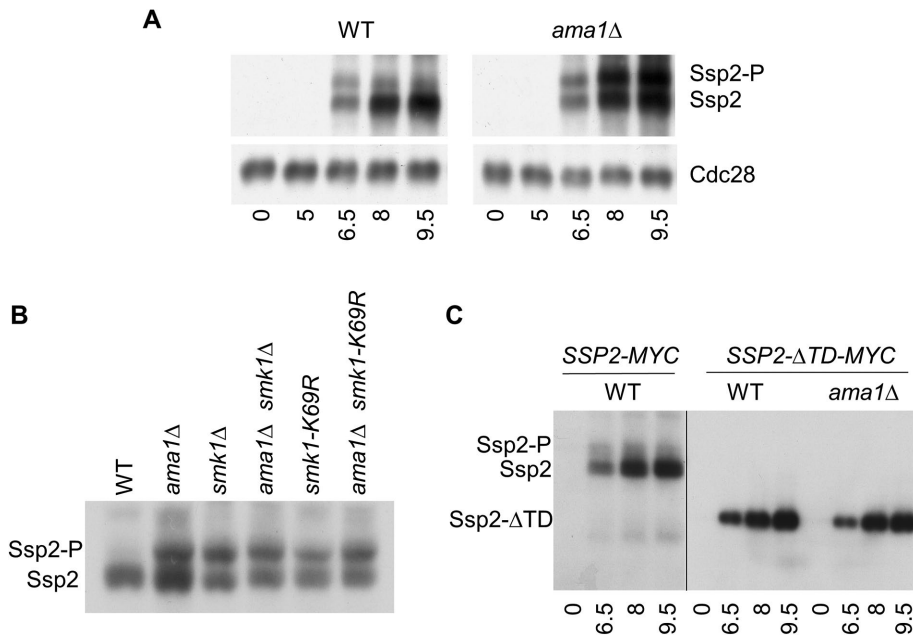


FIGURE 5: Ssp2 is persistently phosphorylated in *ama1Δ* cells. (A) *SSP2-MYC* wild-type and *ama1Δ* cells were incubated in sporulation medium for the indicated times (h), and total protein was analyzed by phostag-acrylamide gel electrophoresis and immunoblot analyses using a Myc antibody (Ssp2) or an antibody that recognizes Cdc28 as indicated. (B) *SSP2-MYC* cells harboring the indicated mutations were incubated in sporulation medium for 9.5 h and analyzed by phostag-acrylamide gel electrophoresis and immunoblot analysis using a Myc antibody. Greater than 80% of cells had completed MII in all cases. (C) *SSP2-MYC* cells and *SSP2-ΔTD-MYC* with and without *AMA1* were collected at the indicated times (h) and analyzed by phostag-acrylamide gel electrophoresis and immunoblot analyses using a Myc antibody.

In this study, we show that, although Ssp2 remains hyperphosphorylated in *ama1Δ* cells, it also remains hyperphosphorylated in *smk1-K69R* cells in which Ssp2 is complexed to Smk1 (Tio *et al.*, 2015, 2017). Further, we have shown that a mutant form of Ssp2 that cannot bind Smk1 is persistently hyperphosphorylated. Moreover, Ama1 controls the binding of Ssp2-ΔTD, which does not appear to be differentially phosphorylated during meiosis. Smk1 is phosphorylated exclusively on its activation loop, and unphosphorylated, singly phosphorylated, and doubly phosphorylated Smk1 can form a complex with Ssp2 (Tio *et al.*, 2017). Thus, although targeting subunits of the APC/C are well known to control cell cycle events by influencing the dephosphorylation of regulatory proteins, these data indicate that Ama1 does not trigger Ssp2/Smk1 binding by promoting the dephosphorylation of either of these proteins. One possibility is that an inhibitory protein in the PSM that is targeted for destruction by Ama1 prevents formation of the Ssp2/Smk1 complex (see Figure 7).

Our data suggest that Smk1 and Ssp2 are destroyed by an *AMA1*-dependent pathway after spore wall assembly has occurred, leading to an absence of Smk1 and a reduced amount of Ssp2 in mature spores. While Smk1 may be recruited to the APC/C by Ama1, it is also likely that the block in meiotic progression observed in *ama1Δ* cells prevents cells from reaching the point in meiotic development at which Smk1 degradation can occur. While most canonical MAPK signaling is opposed by phosphatases that remove phosphate from the activation loop, Smk1 appears to remain fully phosphorylated until it is degraded. These findings suggest that Smk1 signaling is terminated by regulated protein destruction as sporulation draws to a close.

MATERIALS AND METHODS

Yeast strains, culture conditions, and plasmids

All yeast strains in this study were in the SK1 background (Table 1). Cells were grown in vegetative cultures in YEPD (1% yeast extract, 2% peptone, 2% glucose) supplemented with adenine to 40 μg/ml, or SD (0.67% yeast nitrogen base without amino acids, 2% glucose, and nutrients essential for auxotrophic strains) at 30°C. Sporulation assays were performed by inoculating vegetative cells from YEPD into YEPA (1% yeast extract, 2% peptone, 2% potassium acetate) supplemented with adenine to 40 μg/ml and growing them overnight at 30°C to a density of 10⁷ cells/ml. Cells were pelleted by centrifugation, washed, and resuspended in sporulation medium (2% potassium acetate, 10 μg/ml adenine, 5 μg/ml histidine, 30 μg/ml leucine, 7.5 μg/ml lysine, 10 μg/ml tryptophan, 5 μg/ml uracil) at 4 × 10⁷ cells/ml, and incubated in a roller drum at 30°C. Estradiol-inducible *NDT80* cells were sporulated as previously described (Whinston *et al.*, 2013) except that β-estradiol was added to 2 μM at 6 h postinduction. Plasmids used in this study are listed in Table 2. The plasmid pRS314-*SSP2*, was made by ligating a PCR fragment containing the *SSP2* open reading frame with 500 base pairs upstream and 500 base pairs downstream

as a *KpnI/SpeI* fragment into pRS314. pRS314-*SSP2-F307A,F327A* was generated from pRS314-*SSP2* by site-directed mutagenesis. The *SSP2-F307A,F327A-GFP* plasmid was generated from pJ82 by site-directed mutagenesis. Mutated genes in plasmids were sequenced in their entirety to assure that only the desired substitutions were present. Wild-type *SSP2* was replaced with *SSP2-ΔTD-F/A-GST* using the strategy that was described for *SSP2-ΔTD-GST*, except that the source of DNA for the *SSP2* PCR was pRS314-*SSP2-F307A,F327A* instead of wild-type genomic DNA (Tio *et al.*, 2015). The genomic copy of *SSP2-ΔTD-MYC* in EWY196 and EWY198 was generated by replacing the GST segment of *SSP2-ΔTD-GST* with MYC using a previously described strategy (Tio *et al.*, 2015).

Purification of proteins

Purification of GST-tagged proteins was performed as described in Tio *et al.* (2015). Briefly, 1 × 10⁹ cells were collected using centrifugation and resuspended in 1 ml of lysis buffer (300 mM NaCl, 5 mM MgCl₂, 25 mM Tris-Cl, pH 7.4, 0.5% NP-40, and protease inhibitors to the concentrations specified; Schindler and Winter, 2006). The cells were lysed using three 40-s and one 30-s pulse of a Mini-Beadbeater 24 with 1-min cooling on ice in between pulses. A low-speed centrifugation was used to separate the whole-cell extract from the beads, and a 15,000 rpm spin at 4°C for 10 min was used to clear the cell lysate. Cleared lysates were added to 80 μl of glutathione-Sepharose 4B and incubated for 2 h at 4°C with end-over-end rotation. The beads were washed two times with lysis buffer and two times with wash buffer, and the proteins were eluted with 25 mM reduced glutathione in 25 mM Tris HCl (pH 7.4). The eluted proteins were precipitated with trichloroacetic acid (TCA), washed with acetone, and analyzed by gel electrophoresis. Smk1-HH proteins were

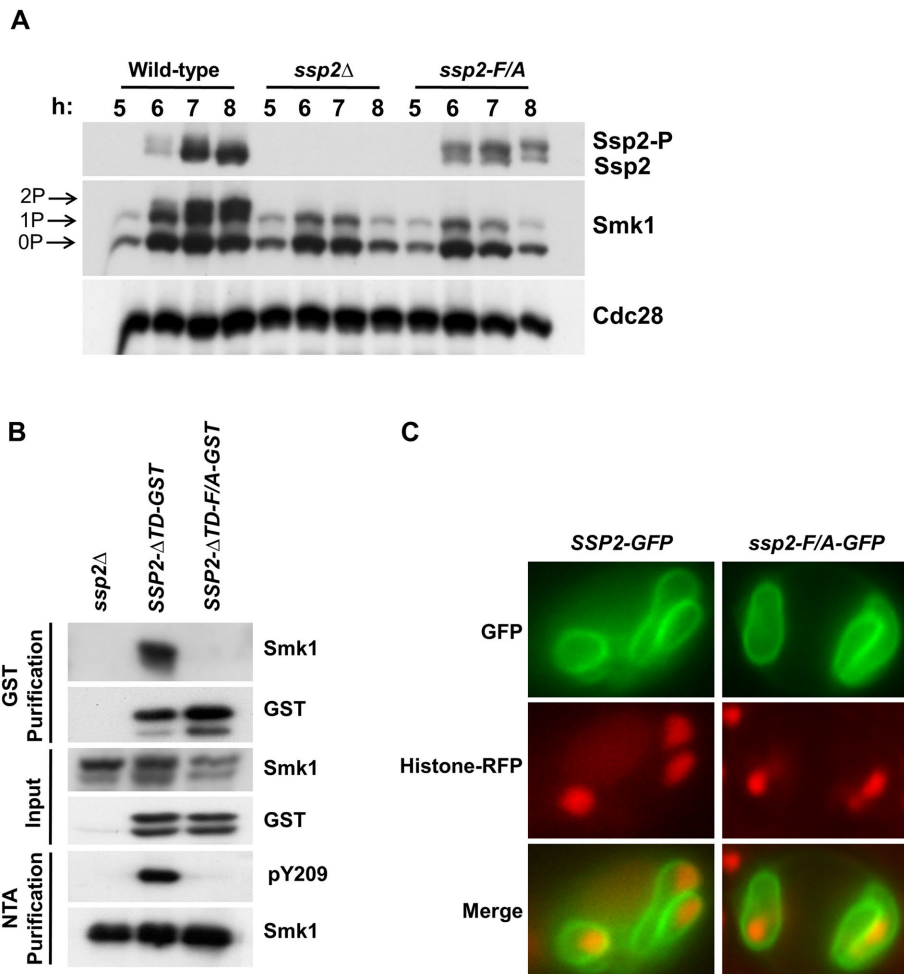


FIGURE 6: A binding-defective Ssp2 mutant is persistently phosphorylated and localized to the PSM. (A) *SSP2-MYC*, *ssp2Δ*, and *ssp2-F/A-MYC* cells were collected at the indicated times after transfer to sporulation medium, and total protein was analyzed by phostag-acrylamide gel electrophoresis and immunoblot analyses using a Myc antibody (Ssp2), an HA antibody (Smk1), or an antibody that recognizes Cdc28 as indicated. (B) *SMK1-HH/SMK1* strains of the indicated *SSP2* genotype (strains TPY11-13) were collected at 9 h postinduction (>80% MII cells in all cases), protein was purified using glutathione resin and assayed with antibodies against HA (Smk1) or GST as indicated. Smk1 autophosphorylation in the same samples was assayed by purifying Smk1-HH using Ni-NTA beads followed by immunoblot analyses with pY209 antisera and an HA antibody. (C) WT cells containing the HTB2-mCherry marker (strain LH902) transformed with multicopy plasmids containing either *SSP2-GFP* or *ssp2-F/A-GFP* as indicated were transferred to sporulation media and viewed by fluorescence microscopy as they completed meiosis.

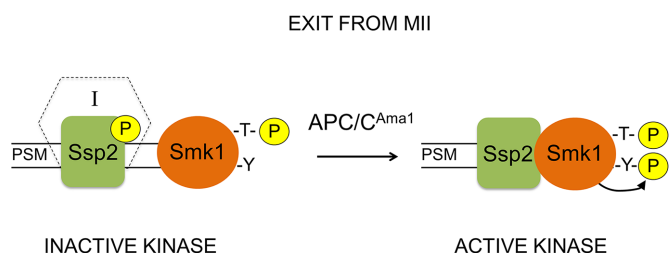


FIGURE 7: Model for the role of Ama1 in the Smk1 pathway. Smk1 and Ssp2 are localized to the PSM during MII (left). At this stage, Smk1 has been phosphorylated on its activation-loop T by Cak1 as shown, but this is insufficient to activate the kinase. Ssp2 is also phosphorylated on at least one residue at this stage, but the identity of the protein kinase(s) is unknown. Upon exit from MII, Ama1

purified under denaturing conditions as previously described (Corbi *et al.*, 2014). In brief, 2×10^8 cells were collected by centrifugation and lysed with NaOH. The proteins were precipitated with TCA, resuspended in denaturing buffer, and purified using nickel beads.

Immunoblot analyses

Sporulating cells were lysed with NaOH, and proteins were precipitated with TCA and solubilized with 8 M urea, as previously described (Knop *et al.*, 1999). This protocol maximizes extraction of membrane-associated proteins from TCA precipitates and has been shown to efficiently extract PSM proteins from sporulating cells. After electrophoresis, proteins were transferred to an Immobilon-P membrane and probed with mouse anti-HA.11 (1:10,000) for Smk1 and mouse anti-MYC (1:5000) and mouse anti-GST (1:500) for Ssp2, as previously described (Tio *et al.*, 2015). Smk1-Y209p analyses were performed as previously described (Whinston *et al.*, 2013). Alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (1:5000) (Promega) was used as a secondary antibody.

Protein samples analyzed using phostag-acrylamide gels were prepared as described above, except that EDTA was omitted from the gel-loading buffer. Samples were electrophoresed through an 8% acrylamide gel containing 50 μ M phostag-acrylamide and 100 μ M MnCl₂. After electrophoresis, the gel was washed in transfer buffer with 2 mM EDTA for 20 min followed by transfer buffer without EDTA. Proteins were transferred to Immobilon-P membranes and analyzed as described above.

Band intensities were quantitated using ImageJ software. For comparison of Ssp2- Δ TD-GST/Smk1-HH binding between wild-type and *ama1Δ* cells, the ratios of the Smk1-HH:Ssp2- Δ TD-GST signals from samples that bound glutathione-Sepharose 4B beads were divided by the ratios from the

corresponding input samples. All immunoblot experiments presented in this study were repeated at least three times, except for the experiments presented in Figure 1, A and C, which were repeated twice.

complexed to the APC/C promotes binding of Ssp2 to Smk1, triggering the autophosphorylation of Smk1's activation loop Y residue. Although Ssp2 is dephosphorylated once the Ssp2/Smk1 complex has formed, Ssp2 dephosphorylation is a consequence of complex formation and does not appear to regulate Ssp2/Smk1 binding. Because complex formation does not appear to be regulated by reversible phosphorylation, we posit that Ama1 targets a repressor (I), shown to interact with Ssp2 for simplicity, for ubiquitin-dependent destruction, thus coupling exit from MII to Smk1 activation.

Strain	Genotype	Source
JTY4	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 his4/his4 ho::LYS2/ho::LYS2 SMK1-HH::LEU2/SMK1-HH::LEU2 ssp2Δ/ssp2Δ</i>	Whinston et al., 2013
JTY5	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 pdr5::KANMX6/pdr5::KANMX6 SMK1-HH::LEU2/SMK1-HH::LEU2</i>	This study
JTY6	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 pdr5::KAN/pdr5::KAN SMK1-HH::LEU2/SMK1-HH::LEU2 ama1::KANMX6/ama1::KANMX6</i>	This study
TPY16	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-HH::LEU2/SMK1-HH::LEU2 SSP2-Δ137-GST::TRP1/SSP2-Δ137-GST::TRP1</i>	This study
TPY15	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-HH::LEU2/SMK1-HH::LEU2 SSP2-Δ137-GST::TRP1/SSP2-Δ137-GST::TRP1 ama1::KAN/ama1::KAN</i>	This study
GOY110	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 his4/his4 ho::LYS2/ho::LYS2 SMK1-HH::LEU2/SMK1-HH::LEU2 ssp2Δ/ssp2Δ ama1::KAN/ama1::KAN</i>	This study
JTY72	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1/SMK1-HH::LEU2 SSP2-ΔN137-GST::TRP1/ssp2::URA3</i>	Tio et al., 2015
LH902	<i>MATa/MATα ho::hisG/ho::hisG lys2/lys2 ura3/ura3 leu2/leu2 his3/his3 trp1ΔFA/trp1ΔFA HTB2-mCherry-TRP1^{C-g}/HTB2-mCherry-TRP1^{C-g}</i>	Parodi et al., 2012
TPY10	<i>MATa/MATα ho::hisG/ho::hisG lys2/lys2 ura3/ura3 leu2/leu2 his3/his3 trp1ΔFA/trp1ΔFA HTB2-mCherry-TRP1^{C-g}/HTB2-mCherry-TRP1^{C-g} ama1::KAN/ama1::KAN</i>	This study
GOY29	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-3HA::HIS3/SMK1-3HA::HIS3 SSP2-13MYC::KANMX6/SSP2-13MYC::KANMX6</i>	Tio et al., 2015
EWY122	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 his4-N and/or his4-G trp1::hisG/TRP1 SMK1-3HA::KANMX6/SMK1-3HA::KANMX6 ssp2Δ/ssp2Δ</i>	Shin et al., 2010
CMY106	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ama1::KANMX6/ama1::KANMX6 ssp2Δ/ssp2Δ SMK1-3HA::Kan/SMK1-3HA::Kan</i>	This study
LRY170	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-3HA::KANMX6/SMK1-3HA::KANMX6 SSP2-13MYC::KANMX6/SSP2-13MYC::KANMX6 ama1::KANMX6/ama1::KANMX6</i>	This study
LRY236	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-3HA::HIS3/SMK1-3HA::HIS3 ssp2-F307A,F327A-13MYC::KANMX6/ssp2-F307A,F327A-13MYC::KANMX6</i>	This study
TPY11	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ssp2-Δ137-F307A,F327A-GST::TRP1/ssp2Δ SMK1-HH::LEU2/SMK1</i>	This study
TPY12	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ssp2-Δ137-GST::TRP1/ssp2Δ SMK1-HH::LEU2/SMK1</i>	This study
TPY13	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ssp2Δ/ssp2Δ SMK1-HH::LEU2/SMK1</i>	This study
JTY27	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 SMK1-3XHA::HIS3/SMK1-3XHA::HIS3 ama1::CaUra3/ama1::CaUra3 clb4::KanMX4/clb4::KanMX4 SCC1p-HA3-NDD1-KanMX4/SCC1p-HA3-NDD1-KanMX4</i>	This study
EWY196	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ssp2Δ137-MYC13-KAN/ssp2Δ137-MYC13-KAN</i>	This study
EWY198	<i>MATa/MATα ura3/ura3 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2 ssp2Δ137-MYC13-KAN/ssp2Δ137-MYC13-KAN ama1Δ::TRP1/ama1Δ::TRP1</i>	This study

All strains are in the SK1 background. The *ssp2Δ* allele in strain JTY4 is deleted for codons 42–242 of the *SSP2* open reading frame and lacks a marker as described (Sarkar et al., 2002).

TABLE 1: Yeast strains.

Microscopy

For assessment of meiotic progression, sporulating cells were fixed with ethanol, stained with 4',6-diamidino-2-phenylindole, and photographed under wet mount using a Nikon Optiphot equipped for epifluorescence as previously described (Krisak et al. 1994). Sporulation efficiency was analyzed by phase-contrast microscopy, with

cells containing two or more spores per ascus scored as positive. For live-cell imaging, it was necessary to use multicopy plasmids to detect the *Smk1*-GFP and *Ssp2*-GFP signals. Cells containing an integrated nuclear *HTB2*-mCherry marker (Table 1) carrying 2 μ -based *SMK1*-GFP or *SSP2*-GFP plasmids (Table 2) were inoculated from selective medium into YEPA for overnight growth and transferred to

Plasmid	Gene/marker	Source
pKC3036	AMA1 + 2 μ TRP1	Tan et al., 2011
pKC3045	AMA1 ^{CBΔ} + 2 μ TRP1	Tan et al., 2011
pKC3046	AMA1 ^{IRA} + 2 μ TRP1	Tan et al., 2011
pKC3048	AMA1 ^{CBΔ/IRA} + 2 μ TRP1	Tan et al., 2011
pJ82	SSP2-GFP + 2 μ LEU2	Sarkar et al., 2002
pJT10	SMK1-GFP + 2 μ LEU2	Tio et al., 2015
pJTb6	SSP2-F307A,F327A-GFP + 2 μ LEU2	This study
pRS314-SSP2	SSP2 + CEN TRP1	This study
pRS314-SSP2-F/A	SSP2-F307A,F327A + CEN TRP1	This study

TABLE 2: Plasmids.

sporulation medium as previously described (Tio et al., 2015). A Leica DM-RXA with oil immersion was used to image 8 μ l of sporulating cells, using a 60 \times lens, at the indicated times. Completion of MI1 was monitored using the HTB2-mCherry marker and further assayed for GFP fluorescence. The fraction of postmeiotic cells that showed GFP fluorescence varied between trials from 8% to 20% due to plasmid loss. All Smk1-GFP and Ssp2-GFP experiments described in this study were repeated independently at least three times with a minimum of 100 fluorescent cells examined.

ACKNOWLEDGMENTS

We thank Katrina Cooper for AMA1 plasmids used in this study. This work was supported by a grant from the National Science Foundation (MCB-0950009 to E.W.) and grants from the National Institutes of Health (R01 GM120090 to E.W. and R01 GM072540 to A.M.N.).

REFERENCES

Ahmed NT, Bungard D, Shin ME, Moore M, Winter E (2009). The Ime2 CDK-like kinase enhances the disassociation of the Sum1 repressor from middle meiotic promoters. *Mol Cell Biol* 29, 4352–4350.

Berchowitz LE, Gajadhar AS, van Werven FJ, De Rosa AA, Samoylova ML, Brar GA, Xu Y, Xiao C, Futcher B, Weissman JS, et al. (2013). A developmentally regulated translational control pathway establishes the meiotic chromosome segregation pattern. *Genes Dev* 27, 2147–2163.

Berchowitz LE, Kabachinski G, Walker MR, Carlile TM, Gilbert WV, Schwartz TU, Amon A (2015). Regulated formation of an amyloid-like translational repressor governs gametogenesis. *Cell* 163, 406–418.

Brar GA, Yassour M, Friedman N, Regev A, Ingolia NT, Weissman JS (2012). High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* 335, 552–557.

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998). The transcriptional program of sporulation in budding yeast. *Science* 282, 699–705 [correction published in *Science* (1998), 282, 1421].

Cooper KF, Mallory MJ, Egeland DB, Jarnik M, Strich R (2000). Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosose in yeast. *Proc Natl Acad Sci USA* 97, 14548–14553.

Cooper KF, Strich R (2011). Meiotic control of the APC/C: similarities and differences from mitosis. *Cell Div* 6, 16.

Corbi D, Sunder S, Weinreich M, Skokotas A, Johnson ES, Winter E (2014). Multisite phosphorylation of the Sum1 transcriptional repressor by S-phase kinases controls exit from meiotic prophase in yeast. *Mol Cell Biol* 34, 2249–2263.

Daubner GM, Clery A, Allain FH (2013). RRM-RNA recognition: NMR or crystallography...and new findings. *Curr Opin Struct Biol* 23, 100–108.

Diamond AE, Park JS, Inoue I, Tachikawa H, Neiman AM (2009). The anaphase promoting complex targeting subunit Ama1 links meiotic exit to cytokinesis during sporulation in *Saccharomyces cerevisiae*. *Mol Biol Cell* 20, 134–145.

Knop M, Siegers K, Pereira G, Zachariae W, Winsor B, Nasmyth K, Schiebel E (1999). Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast* 15, 963–972.

Krisak L, Strich R, Winters RS, Hall JP, Mallory MJ, Kreitzer D, Tuan RS, Winter E (1994). *SMK1*, a developmentally regulated MAP kinase, is required for spore wall assembly in *Saccharomyces cerevisiae*. *Genes Dev* 8, 2151–2161.

Maier P, Rathfelder N, Finkbeiner MG, Taxis C, Mazza M, Le Panse S, Haguenaer-Tsapis R, Knop M (2007). Cytokinesis in yeast meiosis depends on the regulated removal of Ssp1p from the prospore membrane. *EMBO J* 26, 1843–1852.

McDonald CM, Cooper KF, Winter E (2005). The Ama1-directed anaphase-promoting complex regulates the Smk1 mitogen-activated protein kinase during meiosis in yeast. *Genetics* 171, 901–911.

Neiman AM (1998). Prospore membrane formation defines a developmentally regulated branch of the secretory pathway in yeast. *J Cell Biol* 140, 29–37.

Neiman AM (2005). Ascospore formation in the yeast *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 69, 565–584.

Neiman AM (2011). Sporulation in the budding yeast *Saccharomyces cerevisiae*. *Genetics* 189, 737–765.

Oelschlaegel T, Schwickart M, Matos J, Bogdanova A, Camasses A, Havlis J, Shevchenko A, Zachariae W (2005). The yeast APC/C subunit Mnd2 prevents premature sister chromatid separation triggered by the meiosis-specific APC/C-Ama1. *Cell* 120, 773–788.

Okaz E, Arguello-Miranda O, Bogdanova A, Vinod PK, Lipp JJ, Markova Z, Zagoriy I, Novak B, Zachariae W (2012). Meiotic prophase requires proteolysis of M phase regulators mediated by the meiosis-specific APC/C-Ama1. *Cell* 151, 603–618.

Parodi EM, Baker CS, Tetzlaff C, Villahermosa S, Huang LS (2012). *SPO71* mediates prospore membrane size and maturation in *Saccharomyces cerevisiae*. *Eukaryot Cell* 11, 1191–1200.

Paulissen SM, Slubowski CJ, Roesner JM, Huang LS (2016). Timely closure of the prospore membrane requires *SPS1* and *SPO77* in *Saccharomyces cerevisiae*. *Genetics* 203, 1203–1216.

Penkner AM, Prinz S, Ferscha S, Klein F (2005). Mnd2, an essential antagonist of the anaphase-promoting complex during meiotic prophase. *Cell* 120, 789–801.

Pesin JA, Orr-Weaver TL (2008). Regulation of APC/C activators in mitosis and meiosis. *Annu Rev Cell Dev Biol* 24, 475–499.

Pierce M, Wagner M, Xie J, Gailus-Durner V, Six J, Vershon AK, Winter E (1998). Transcriptional regulation of the *SMK1* mitogen-activated protein kinase gene during meiotic development in *Saccharomyces cerevisiae*. *Mol Cell Biol* 18, 5970–5980.

Sarkar PK, Florczyk MA, McDonough KA, Nag DK (2002). *SSP2*, a sporulation-specific gene necessary for outer spore wall assembly in the yeast *Saccharomyces cerevisiae*. *Mol Genet Genomics* 267, 348–358.

Schindler K, Winter E (2006). Phosphorylation of Ime2 regulates meiotic progression in *Saccharomyces cerevisiae*. *J Biol Chem* 281, 18307–18316.

Shin ME, Skokotas A, Winter E (2010). The Cdk1 and Ime2 protein kinases trigger exit from meiotic prophase in *Saccharomyces cerevisiae* by inhibiting the Sum1 transcriptional repressor. *Mol Cell Biol* 30, 2996–3003.

Tan GS, Lewandowski R, Mallory MJ, Strich R, Cooper KF (2013). Mutually dependent degradation of Ama1p and Cdc20p terminates APC/C ubiquitin ligase activity at the completion of meiotic development in yeast. *Cell Div* 8, 9.

Tan GS, Magurno J, Cooper KF (2011). Ama1p-activated anaphase-promoting complex regulates the destruction of Cdc20p during meiosis II. *Mol Biol Cell* 22, 315–326.

Tio CW, Omerza G, Phillips T, Lou HJ, Turk BE, Winter E (2017). Ssp2 binding activates the Smk1 mitogen-activated protein kinase. *Mol Cell Biol* 37, e00607-16.

Tio CW, Omerza G, Sunder S, Winter E (2015). Autophosphorylation of the Smk1 MAPK is spatially and temporally regulated by Ssp2 during meiotic development in yeast. *Mol Biol Cell* 26, 3546–3555.

Torres MP, Borchers CH (2007). Mitotic phosphorylation of the anaphase-promoting complex inhibitory subunit Mnd2 is necessary for efficient progression through meiosis I. *J Biol Chem* 282, 17351–17362.

Wagner M, Pierce M, Winter E (1997). The CDK-activating kinase Cak1 can dosage suppress sporulation defects of *SMK1* MAP kinase mutants and is required for spore wall morphogenesis in *Saccharomyces cerevisiae*. *EMBO J* 16, 1305–1317.

Whinston E, Omerza G, Singh A, Tio CW, Winter E (2013). Activation of the Smk1 mitogen-activated protein kinase by developmentally regulated autophosphorylation. *Mol Cell Biol* 33, 688–700.